REMARKS/ARGUMENTS

In the Notice dated June 17, 2009, the Examiner noted that claim 4 had been amended to delete reference to "lactoferrin" and include the limitation "lactofenin" and therefore the proper status of the claim should be "Currenly Amended". Applicants respectfully submit that inclusion of the term "lactofenin" in claim 4 was an inadvertent typographical error. The correct term is lactoferrin, as recited in the originally filed claims. Accordingly, Applicants have corrected the typographical error (i.e., "lactofenin" has been replaced with "lactoferrin") in claim 4 and maintained the status identifier of claim 4 as "Original". Applicant submits that the amendment is now compliant and respectfully requests entry of the amendments submitted herewith. Applicant's arguments submitted in the Amendment dated March 23, 2009 are also reiterated herein below

Claims 1-17 are currently pending in this application and claim 18 has been canceled, all of which were rejected. Applicants have amended the specification to properly identify the trademarks recited therein capitalizing the trademark recitations and no new matter has been added. Claims 1 and 17 have been amended to recite that the Lipopolysaccharide (LPS)-protein complex is of a Lipopolysaccharide (LPS) and a protein to provide antecedent basis for the terms "the Lipospolysaccharide (LPS)" and "the protein" in the claims. Claim 1 has also been amended to correct typographical errors. Claim 7 has been amended to provide proper reference to "the" LPS. Claim 17 has also been amended to recite that the claimed process comprises affixing the LPS of the complex to an anion exchange chromatographic resin. Support for the amendments to claim 17 can be found in claim 18 as originally filed. Claim 18 has been cancelled. Reconsideration and allowance of the claims is respectfully requested in view of the foregoing amendments and the following remarks.

1. Objection to the Specification.

The Examiner has objected to the specification because the use of trademarks has been noted and according to the Examiner the trademark recitations should be capitalized wherever they appear. In response applicants have amended the specification to properly identify the trademarks recited therein, capitalizing the trademark recitations throughout the specification. No new matter has been added by these amendments to the specification. Accordingly, applicants respectfully request withdrawal of the objection to the specification.

2. Claims 1-18 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite.

Claims 1-18 are rejected under 35 U.S.C. 112, second paragraph as allegedly being indefinite. According to the Examiner the term "a portion of the LPS" and "a portion of the protein" in claims 1 and 17, and the term "a portion of the endotoxin" in claim 14 renders the claims indefinite. The examiner asserts that it is unclear what constitutes a portion and how much of the original structure of the LPS, protein, or endotoxin has been retained. In response applicants submit that the term "a portion of the LPS/protein/endotoxin" refers, in contrast to the Examiner's assertions, not to a part of the structure of the LPS, protein or endotoxin but rather refers to a part of the entire amount (a portion) of the LPS, protein, or endotoxin present. This is also made clear to the skilled artisan reading the specification in the recitations on page 7, line 13 to page 8, line 2 and page 8, lines 7-18 of the specification. These paragraphs clearly discuss a portion of the LPS/protein/endotoxin as a relative amount of the total amount of the LPS/protein/endotoxin. Accordingly, applicants submit that the terms "a portion of the LPS", "a portion of the protein", and "a portion of the endotoxin" clearly define the claimed subject matter as understood by the skilled artisan.

The Examiner asserts that in claim 1, it is unclear how the terms "an endotoxin" and "a Lipopolysaccharide" differ from each other in terms of scope. The Examiner also asserts that in the recitation "LPS or other endotoxin" in claim 7 it is unclear what is encompassed by the term "other endotoxin." In response applicants submit that although the terms endotoxin and Lipopolysaccharide may frequently be used interchangeably, the term endotoxin refers to a broader group of bacterial toxins and Lipolysaccharides constitute a subset thereof. The skilled artisan would understand that the terms "the LPS" and "endotoxin" may represent different toxins where LPS represents a subset of endotoxins. Accordingly, applicants submit that the recitations of either Lipolysaccharide or endotoxin in claim 1, or "LPS or other endotoxin" in claim 7 clearly define the claimed subject matter.

According to the Examiner, claims 1 and 17 do not provide proper antecedent basis for the terms "the LPS" and "the protein" in the claims considering only "a Lipopolysaccharide(LPS)-protein complex" is recited in an earlier recitation. In response applicants have amended claims 1 and 17 to recite that the Lipopolysaccharide (LPS)-protein complex is of a Lipopolysaccharide (LPS) and a protein to provide antecedent basis for the later recitation of "the Lipospolysaccharide (LPS)" and "the protein" in the claims.

Accordingly, applicants submit that the amendments to claim 1 and 17 more clearly define the claimed subject matter.

Claim 7 is indefinite according to the Examiner because it is unclear whether the term "LPS" in claim 7 refers to "the LPS" as in claim 1. In response applications have amended claim 7 to recite "the LPS" to more clearly define the claimed subject matter.

The Examiner asserts that claim 11 is indefinite because of the term "high salt environment" considering that the term "high" is a relative term. In response applicants submit that the claimed processes relate to separating (eluting) either the LPS, endotoxin, or protein from a (chromatographic) resin. In the art of removing material of biological origin (either LPS, endotoxin, or protein) from a chromatographic resin, in particularly in obtaining (recombinant) proteins from a bacterial expression system, the skilled artisan would immediately understand the meaning of the term "high salt environment" in this context. Accordingly, applicants submit that claim 11 clearly defines the claimed subject matter.

Claims 8, 13, and 17 are rejected as vague and indefinite because according to the Examiner the terms "changing the pH" or "a change in pH or conductivity" are unclear as to the pH or conductivity of what is being changed and from which pH (or conductivity) to which pH (or conductivity) the change is made. In response applicants submit that the claimed processes relate to separating (eluting) either the LPS, endotoxin, or protein from a (chromatographic) resin. In the art of removing material of biological origin (either LPS, endotoxin, or protein) from a chromatographic resin, in particularly in obtaining (recombinant) proteins from a bacterial expression system, the skilled artisan would immediately understand the meaning of the terms "changing pH" or "a change in pH or conductivity" in this context as is also made clear in the specification on page 8, lines 3-6. Accordingly, applicants submit that claims 8, 13, and 17 clearly defines the claimed subject matter.

For these reasons applicants submit that claims 1-17 clearly define the claimed subject matter (as understood by the skilled artisan). Claim 18 has been canceled and any rejections of this claims are thus now moot. Withdrawal of the rejections under 35 U.S.C. 112, second paragraph is respectfully requested.

3. Claims 1-3, 5, 6, 8-10, 11, 14, 15, 17, and 18 are rejected under 35 U.S.C. § 102(e), as allegedly being anticipated.

Claims 1, 3-5, 6, 8-10, 11, 14, 15, 17, and 18 are rejected under 35 U.S.C. 102(e) as allegedly being anticipated by Hauser et al (US 6,966,992), in light of Petch et al (*J. Biotechnol.* 76: 97-119, 2000) as set forth on pages 5 and 6 of the Office Action. According to the Examiner, Hauser et al disclosed a method of purifying proteins by recombinant DNA techniques from its impurities including carbohydrates and lipids using a chromatographic resin and a buffer containing non-flammable, cheaper and less denaturing solvent, 1,6-hexanediol, 1,5-pentadiol, or 1,7-heptanediol. The Examiner asserts that the process in Hauser et al inherently serves as a process of removing an endotoxin from the mixture of polypeptides from endotoxin containing recombinant Gram negative *E. Coli* cells, because Petch et al taught that endotoxin is a common impurity in recombinant protein solutions from recombinant *E. Coli* that is removed chromatographically.

In response applicants submit that as mentioned in the description of the present patent application, lipopolysaccharide (LPS) is a major component of the outer membrane of gram negative bacteria. The endotoxic component of LPS is the lipid A portion. It comprises 1,6-linked glucosamine residues that are substituted with up to six acyl chains and a core polysaccharide structure to which additional polysaccharide repeating units may be attached. Endotoxin is a potent activator of the innate immune system at low doses while at higher doses endotoxin induces a number of other physical reactions including septic shock and death. Contamination of therapeutic products with endotoxins is therefore a primary concern for the manufacturers of such products.

Many recombinant proteins are produced in the gram negative bacteria *Escherichia coli*. The removal of LPS from these recombinant proteins can be a complicated but essential process especially if the proteins are destined for therapeutic uses. Often, during the production of recombinant proteins, difficulties in the separation of LPS from proteins are encountered due to protein-LPS interactions.

The claimed invention is directed to a process for removing an endotoxin from recombinantly produced proteins comprising a Lipopolysaccharide (LPS)-protein complex comprising the steps of:

immobilizing the complex to a chromatographic resin;

washing the resin with an alkanediol whereby at least a portion of the LPS is separated from the complex; and,

eluting at least a portion of the protein from the resin.

This results in effectively removing at least a portion of the LPS from the protein.

Hauser et al. discloses a method of purifying molecules, in particular proteins and peptides, from a mixture (abstract + column 2, lines 27 to 28). The mixture may contain a number of biological components such as carbohydrates, lipids, proteins, and nucleic acids (column 6, lines 40 to 42). First, the mixture is loaded onto a reverse phase liquid chromatographic column. Second, the molecule is **eluted** from the column with a buffer containing a diol, which is 1,5-pentanediol, 1,6-hexanediol, or 1,7 heptanediol (column 2, lines 28 to 32).

The fact that Hauser et al. mentions that carbohydrates and lipids may be present in the mixture is not equivalent to a disclosure nor suggests that the endotoxic component of LPS might be present in the mixture as well. For this reason alone a Lipopolysaccharide (LPS)-protein complex is not disclosed by Hauser et al.

Furthermore, loading and **washing** steps in the process according to Hauser et al. are carried out with buffers which do not contain any alkanediol. It is only when **eluting** the desired molecule that a diol is used. Petsch et al. discloses that endotoxin may be present as a contaminant in protein solutions in view of the remarkable capability of endotoxin to interact with proteins (page 105, §3.4). Accordingly, in light of Petsch et al. it can be suggested that the protein disclosed by Hauser will inherently contain some endotoxin. However, by **eluting** the protein from the chromatographic resin with a buffer comprising a diol, the resulting protein will still contain the same amount of endotoxin.

It has not been disclosed by Hauser et al. that by immobilizing the Lipopolysaccharide (LPS)-protein complex to a chromatographic resin and subsequently **washing** the resin with an alkanediol at least a portion of the LPS will be separated from the complex. Therefore, Hauser et al fails to disclose the step of washing the resin with an alkanediol whereby at least a portion of the LPS is separated from the complex as is required by the claimed invention.

Thus, the present claims 1-3, 5, 6, 8-10, 11, 14, 15, and 17 are not anticipated by Hauser et al. in light of Petsch et al. With respect to claim 18, applicants have canceled this claim and therefore the rejection with respect to this claim is now moot. For these reasons, applicants respectfully request withdrawal of the rejections under 35 U.S.C. 102(e).

4. Objection to claim 1.

The Examiner objected to claim 1 for including the incorrect term "an" in "an chromatographic resin." Applicants have amended the recitation in claim 1 to "a

chromatographic resin" to correct the typographical error. Accordingly, withdrawal of the objection to claim 1 is respectfully requested.

It is believed that claims 1-17 are now in condition for allowance, early notice of which would be appreciated. If any outstanding issues remain, the examiner is invited to telephone the undersigned at the telephone number indicated below to discuss the same.

CONCLUSION

If the undersigned can be of assistance to the Examiner, please contact the undersigned at the number set forth below. In the event the United States Patent and Trademark Office determines that an additional extension and/or other relief is required, Applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with this filing to Deposit Account No. 50-4205; Reference Number: 2003.795US.

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Schering-Plough Corporation
2000 Galloping Hill Road
K-6-1-1990
Kenilworth, New Jersey 07033-0530
Tel: (908) 298-7486
Fax: (908) 298-5388

Respectfully submitted,

Carol M. Gruppi
Attorney for Applicants
Reg. No. **37,341**